Atty Dkt. No. 112461-016

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application of:	)
Joanne Y. H. Kwak-Kim et al.	)
For: DIAGNOSIS AND TREATMENT OF INFERTILITY	)
Serial No. 10/651,690	)
Filed: August 28, 2003	)
Examiner: Michael E. Szperka	)
Art Unit: 1644	)
Conf. No. 9043	)

CERTIFICATE OF MAILING

I hereby certify that this paper is being deposited with the United States Postal Office with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on May 23, 2006.

Tin-Chuen Yeung

COMBINED DECLARATION OF JOINT INVENTORS UNDER 37 C.F.R. §131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Joanne Young Hee Kwak-Kim, M.D. and Alice Gilman-Sachs, Ph.D. aver as follows:

1. We are over the age of twenty-one years and make these statements from our own personal knowledge.

2. I, Dr. Kwak-Kim currently hold the position of the Assistant Chair, Department of Obstetrics and Gynecology; and the Medical Director, the Clinics at Rosalind Franklin University of Medicine and Science; and the Director, Women's Health Division, University Clinics; and Associate Professor, Department of Obstetrics and Gynecology and the Department of Microbiology and Immunology of the Rosalind Franklin University of Medicine and Science (formerly known as Finch University of Health Sciences)/The Chicago Medical School.

3. I, Dr. Gilman-Sachs, currently hold the position of Associate Professor of the Rosalind Franklin University of Medicine and Science (RFUMS) and also hold the position of Associate Director Clinical Immunology Laboratory for RFUMS.

4. We are both joint inventor of the above-captioned patent application.

5. Joint inventor Alan E. Beer is deceased.

6. Prior to April 19, 1999 we planned to study the affect on reproductive outcomes, in subjects with a history of recurrent spontaneous abortions or implantation failures, by adjusting the balance of T helper 1 (Th1) and T helper 2 (Th2) immune responses in the subject. A letter signed by Dr. Kwak-Kim with the date expurgated is attached as Exhibit 1 and was mailed prior to the Critical Date. In particular, we determined to decrease the ratio of Th1 immune response to Th2 immune response by either (a) down regulating the Th1 immune response, (b) by up regulating the Th2 immune response or (c) by both down regulating the Th1 immune response while up regulating the Th2 immune response.

7. Further to this planned study, prior to the Critical Date we began development of an assay to measure the ratio of Th1 to Th2 immune responses in a subject. We have attached as Exhibit 2 a set of laboratory notebook pages with dates removed evidencing the development of the assay. The ratio of the Th1 to Th2 immune responses can be measured by absolute cell counts or percentage of Th1 cells to Th2 cells. Th1 cells are the activated T-cells expressing Th1 cytokines such as IL-1, IL-2, IFN-γ and TNF-α. Th2 cells are the activated T-cells expressing Th2 cytokines such as IL-4, IL-5, IL-6 and IL-10. The ratio of the Th1 to Th2 immune responses can also be determined by calculating a ratio of any one of the Th1 cytokines to any one of the Th2 cytokines.

8. One method we contemplated to reduce the Th1 count was to administer to a subject, prior to conception by the subject, a TNF-α antagonist. TNF-α antagonist may be of several types including antibodies, soluble receptors, and chemical compounds. We contemplated using several commercially available TNF-α antagonists and TNF-α antagonists that were undergoing an FDA approval process in the hope of becoming commercially saleable. Examples of antibody type and soluble receptor-type TNF-α antagonists included, but were not limited to: (1) infliximab (antibody-type) (2) entanercept (soluble receptor-type) (See Exhibit 1), (3) D2E7 (antibody-type) (4) CDP571 (antibody-type) and (5) CDP870 (antibody-type).

9. We contemplated administering the TNF-α antagonist by any medically suitable route of administration.

10. After conceiving of these concepts we worked on them diligently from prior to the Critical Date up to the time of filing the above-captioned patent application.

11. All of the work we have referred to herein was done in the United States of America.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, I acknowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and may jeopardize the validity of the application or any patent issuing thereon.

Date: 5/17/2006 BY _____

Dr. Joanne Kwak-Kim

Date: 5/17/2006 _____

Dr. Alice Gilman-Sachs

EXHIBIT 1

Mr. Richard McKenna
Medial Scientist Liaison
Wyeth-Ayerst Laboratories
15060 Hale Drive
Orland Park, IL 60462

Dear Mr. McKenna:

Thank you for your prompt response. I was glad to hear that your company had an interest in possible anti-TNF application for women with recurrent spontaneous abortions and infertility of immune etiology. I am sending some of our research articles and patient education materials for your perusal. You may find other information in our web site, repro-med.net.

I am preparing my idea for a possible clinical study using etanercept. Hopefully we can conduct a nice clinical trial in future.

If you have any questions, please feel free to contact me at any time.

Sincerely,

Joanne Kwak-Kim, M.D.

Joanne Y. H. Kwak-Kim, M.D.
Associate Director, Reproductive Medicine
Assistant Professor, Department of Obstetrics and Gynecology
Assistant Professor, Department of Microbiology and Immunology

EXHIBIT 2

Sigmaplot

~~X~~ ~~select the return~~

① statistics

② Regression wizard

③ Sigmoidal ~~3 parameter~~

④ Logistic 4 parameter

(if higher parameter does not work, use lower one (3))

⑤ Parameters : First empty need in report ☑
create new graph ☑

↓

Finish.

* title on column

ID

type

* Color list sim name change

Rt click : Edit click

→ type new name

1) ~~IgG~~ 1mg/ml 50ul 1hr
2) Block 200ul 1% BSA
3) Enz anti-~~IgG~~ 50ul
   Substrate 50ul

50ul

(5)
(ml)
1 ~ 1000 ml
0.1 ~ 100
0.01 ~ 10
0.005 ~ 5ml
(4.995)

IgG (4g dilution)

1:10 1:10 1:100 1:100 1:500 1:500 1:1000 1:1000

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	IgG 1mg	1:10	1:100	1:100							
B			1:10	1:10								
C			1:10	1:10								
D			1:10	1:10								
E			1:10									
F			1:10									
G												
H												

1:1000
"
1:2000 + 1ml PBS
"
1:4000
"
2ml + 2ml
0.1% BSA-TBS

50ul
12x 0.05ml (1ml) PBS
.05
.60
1:10 1ml
1:100 1ml
1:500 1ml
1:1000 1ml 0.1ml (100) + 0.9ml

0.2ml 1.8ml
0.1ml + 0.9ml PBS
50ul + 450ul
0.2ml + 0.8ml

0.9ml

1:10 / 1:10
:100
0.1 x 0.9
(0.2ml + 1.8ml)

1ml = 1000ul
0.2ml = 200ul

	1	2	3	4	5	6	7	8	9	10	11	12
A	3kof											
B												
C												
D												
E												
F												
G												
H												

+ Substrate (pick one)

1) Antigen — 1hr to overnight in bicarbonate buffer of H.S.O (sticky)

2) Block w add blocking reagent 1% BSA and PBS (phosphate buffered saline) ↳ 1 hr wait

3) Add Antibody (human serum) ↳ 1 hr wait

4) Block.

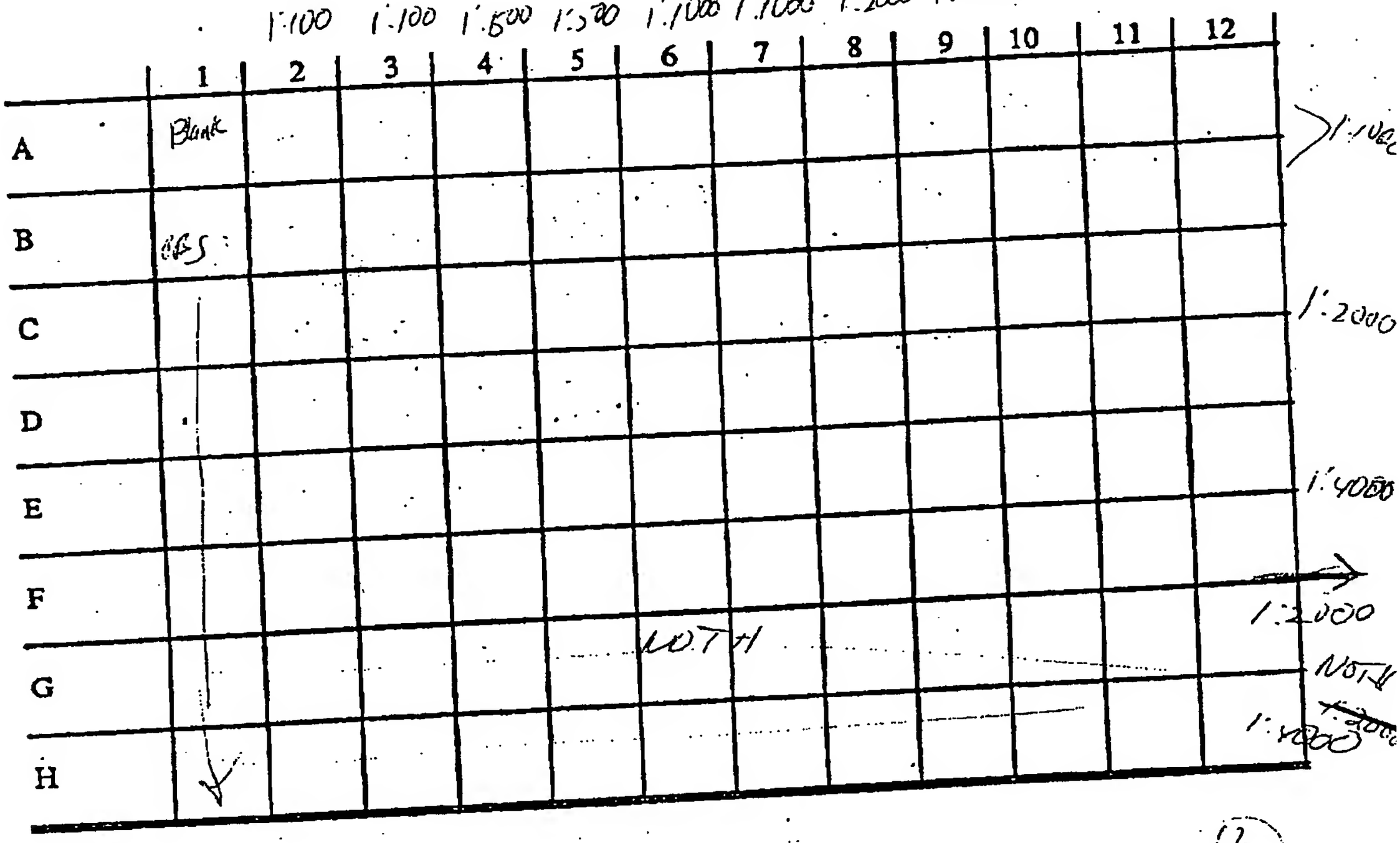
5) Add indirect enzyme conjugated Ab ↳ 1 hr wait

6) Wash → Added Substrate

Ag { 1:100 (bicarbonate) ↓ 20 µl + 2 ml
1:500 (") ↓ 200 µl + 0.8 ml
1:1000 (") 200 µl of 1:100 + 1.8 ml bicarbonate
1:2000 (") 0.05 ml (50 µl of 1:100) + 1.0 ml bicarbonate

human IgG in bicarb

1:100 1:100 1:500 1:500 1:1000 1:1000 1:2000 1:2000



Ag { × 1:100 → .1 × 10.0
.01 → 1 ml
20 µl + 2 ml
12 × 0.05 ml = .60 ml
(12 × 0.05 = 0.60)

× 1:500 1:5 dil = 1:500 0.1 ml × 0.5
0.2 ml × 0.8

1:1000 dilⁿ 0.1 ml + 0.9 ml (0.2 ml × 1.8)

1:2000 dilⁿ ⇒ 1:20 dilⁿ 1 → 20 or .1 → 2.0

.1 → 2.0 ml
0.05 → 1 ml

0.05 → 1.0 ml
(1:20)

① Eliza : basic concept.

Ag (thyroglobin)

Block + Pt's Serum (Anti Ab to Thyroglobin)
(1% Bovine Serum albumin)

Ig (thyroglobin) (Ag)

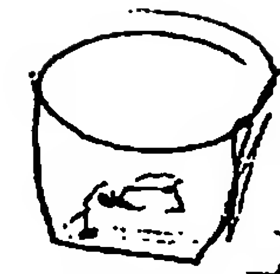
Anti Ab

1:50 dilution
- 1ml → 50 ml
- 0.1ml → 5 ml
- 0.01ml → 0.5ml
- (10ul → 500ul)

(goat anti human IgG: Ap: Alkaline Phosphatase)

(human)

(2)



IgG

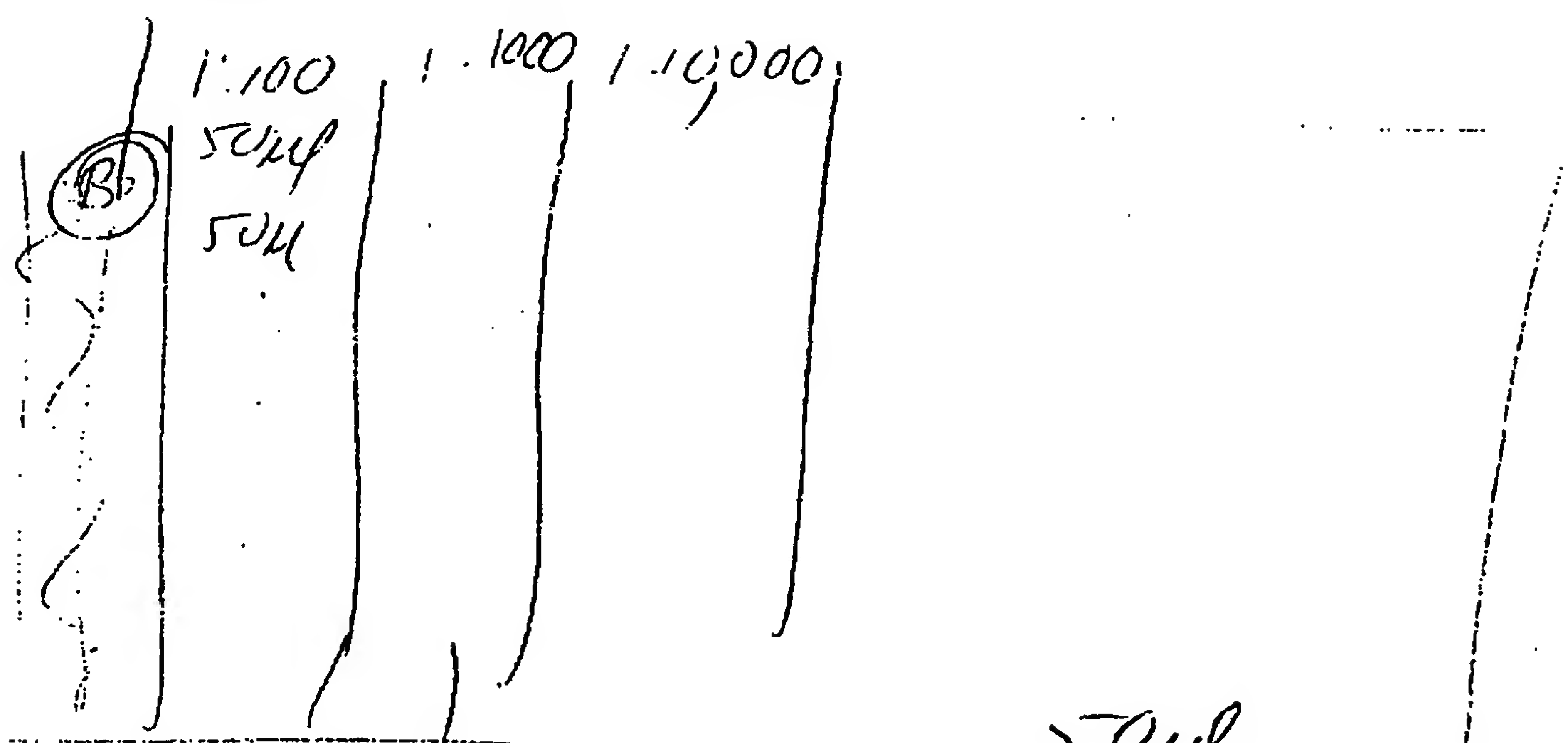
000 µg/ml in PBS

1: 100 ̄c Bicarbonate buffer.

1: 1000

1: 10,000

Bicarb 50 µl



50µl
Leave on 1 Hr RT
Wash 4x ̄c PBS-tween 20 0.05% 200µl
Block with 1% BSA 1 hr (200µl)
Dump
Add ~~anti~~ AP. anti-human IgG in 0.1% BSA (50µl) 1hr
Wash 4x ̄c PBS-Tween 20 (200µl)
Add substrate (50µl) 30min 37°C
Add stopping reagent (optional)
Read OD.

1ml : 199 ml PBS

① anti – CD Ab ( ) mg/ml
→ dilute 1:200 in PBS [No BSA, No Serum],
place 30 ul in each well for stimulation.

② Incubate 1 hr at 37°C or
Overnight at 4°C (refrigerator)

③ Wash cells 2 x with 200 ml PBS – tap out on paper towel (should be sterile)

④ * No stim cells at least 1 away from stim

Stim		N
●	○	○
●	○	○
●	○	○
●	○	○

⑤ 200,000 cells/well in 200ml
(= 1 x 10⁶/ml)

⑥ for flow, Centrifuge in regular tubes, put supernatant into eppendorf.

⑦ collect S/N at 24 hrs, centrifuge in microfuge and place in a fresh tube

⑧ Assay by ELISA immediately or
freeze S/N ≤ -20°C

if multiple ELISA; Aliquot S/N.

– Read ELISA protocol ahead of time
How much sample do you need?

IL-10 1:10 → 100 ul

PHA ± αCD3] compare
3H thymidine − αCD3

PBMC ← Stim Anti CD3

⊛ R&D Systems
DO NOT USE BIOSOURCE (RE?)

IL-2 ↓ (IFNγ) TH1
IL-1α ↑ (TNFα)

anti CD3 10 ug/ml

P+ (10 ml women
: 6 preg women

anti CD3 2 mg/ml (1 mg/ml)
in PBS (NO BSA NO SERUM)

① dilute 1:200, place 30µl in each well for Stimulation

② incubate (1 hr at 37°C or overnight at 4°C (refrig.)

③ wash wells 2x with 200µl PBS - tap out on paper towel. (sterile)
media

④ 200,000 cells/well in 200µl = 1×10⁶/ml → 4 hr incubation (37°C CO₂)

* no stim wells at least 1 away from stim

5a for flow, c. fuge in regular tubes, put S/N into eppendorf

⑤ collect S/N at 24 hrs, centrifuge in microfuge and place in a fresh tube.
S. stim
→ collect the supernatant & Eppendorf pipette

⑥ assay by ELISA immediately or

freeze S/N ≤ -20°C
if multiple ELISA's, aliquot S/N

- read ELISA protocol ahead of time
HOW MUCH SAMPLE DO YOU NEED?

Coulter Epics (Turn On)

① Computer Power On → (wait 2 min)

② 맨 왼쪽 Item (가운데 있는 box) - orange line 있는
- waste box check - $\frac{1}{2}$ 이상이면 dump
- (2 white bottle - dry 대신
  2 transparent bottle - $\frac{1}{3}$ 이하 dry
- Empty 있으면 → 비움

③ Panel → Select → start up click & drag click

④ 맨 밑에 Run 눌러 green blank 되면
open the door (주의! between)
→ Isoflow 채운
button 2-3번 눌러 bubble 없는 check

① 왼쪽 Error message - check
Reagent Empty - check

② Carrosel 의 3개 tube
① Water 1 and 2
② F-check : to drop
③ F-set : to drop

③ 맨 밑 Run 눌러 initialization 하는 동안 맨 왼쪽
⑤ Insert tube 하면 화면 변함
Okay click → 5-6분 Wait

④ Flow-check 할 때 : ||| |||| || HPCV = CV
Flow-Set " same MnIX = Norm CH
MnX = Peak CH Copy 함

⑥ Protocol → Select.

(
Arg 로 Listmodel
model = create
color dialog
||
File_
↓
Fox File
box의 text item 부분에 입력된 숫자 사용
작업시트로.
(if Modal button 누르면 msg → If check 안 됨)

Fox file.
D:\bf dbase 폴더에 있어야 함)
C:\XL\DATA\CUS.Dbf

(다른 printer로 Data는 같이 출력)

Listmodel [Runtime Printer]

shutdown
① water
② ~~sol~~ bleach
③ water
④ water
} about 1 ml

→ Panel
→ select
→ shut down
→ Run
(Manual drain)

take
(take 8-10 min)

put the water 2X
black tube on
(양쪽 다 2번)

Push button
→ green → it will be blinking
in manual talk

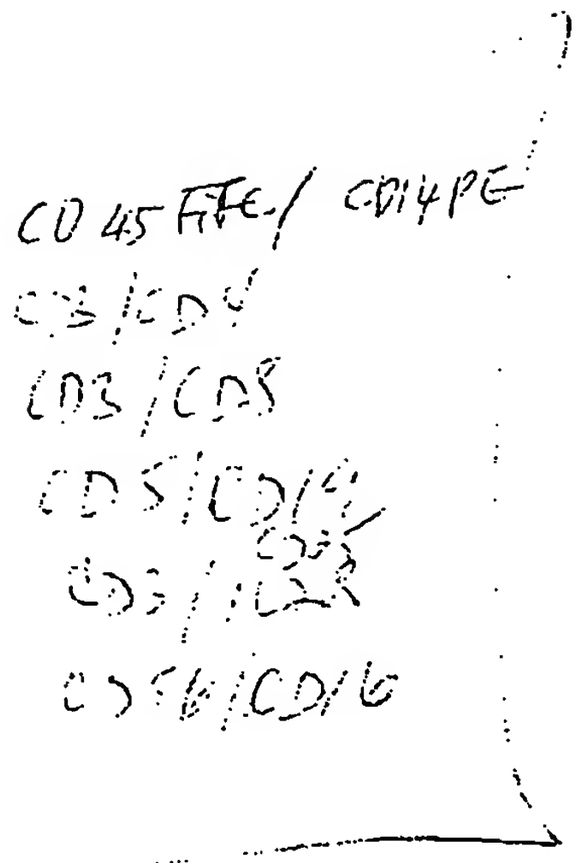
green + blinking → I can open the door
(이때 약간 기울이면서 열어)

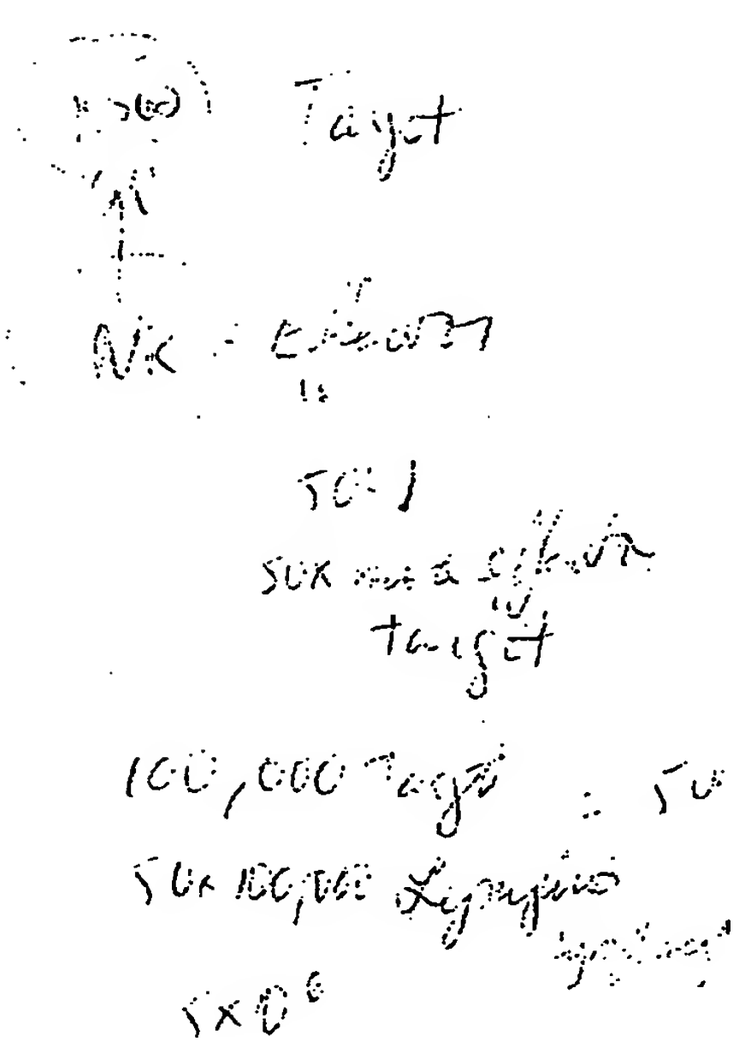
→ take out test tube
→ black tube 넣은
→ 넣은 black tube 넣은

→ test tube 다시 넣은
Auto mode procedure
put 2 tube water
cancel 이 넣은

① . ②

→ Start → 종이비 날림
Pause


CD45RA/CD14PE
CD3/CD4
CD3/CD8
CD5/CD19
CD3/IL2R
CD56/CD16
Cytokines
IL-1
IL-2
IL-3
IL-20


Target
NK - Effector
TCR
SCK mediated target
100,000 Target : TCR
SCK 100,000 Lymphocytes
1X10^6
2.5X100,000
2.5X10^6
Ex. of
Kill
Progidium
iodide
DNA
Sheep RBC
Sheep RBC
1000
400
1500
100
10%
E:T
E:T
E:T
10^6
5/0
5 1/2
Other
Mechanisms
Killing